TABLE 2

Bacteria from maple tapholes (1955 season)

Group, genera and species	Number of cultures	Percent of group
I. Gram negative rods (314;67%) [1]		
Pseudomonas		
Ps. geniculata	167	53.2
Ps. putrefaciens	27	8.6
Ps. mephitica	8	2.5
Ps. striata	9	2.9
Miscellaneous	12	3.8
Flavobacterium		
Fl. solare	36	11.5
Miscellaneous	10	3.1
Achromobacter		
A. superficiale	9	2.9
Miscellaneous	27	8.6
Unclassified	9	2.9
II. Gram positive cocci (78;16.6%)		
Micrococcus		
M. varians	18	23.0
M. luteus	15	19.2
M. conglomeratus	9	11.5
M. candidatus	7	9.1
Miscellaneous	24	30.8
Sarcina	5	6.4
III. Gram positive, non-spore forming rods [2] (52;11.1%)		
IV. Gram positive, spore forming rods (25; 5.3%)		
Bacillus		
B. cereus	12	48.0
B. circulans	10	40.0
Miscellaneous	3	12.0
Total: (469; 100%)		

[1] Number of cultures in group and percent of total number.
[2] This was a very heterogeneous group of cultures.

the largest group was comprised of the psychrophilic, gram negative, rod-shaped bacteria of the genera *Pseudomonas, Flavobacterium,* and *Achromobacter. Pseudomonas geniculata* accounted for more than 50% of the cultures in this group, and for about 37% of all the bacteria isolated. The second largest group was comprised of micrococci with at least 4 species of *Micrococcus* and one species of *Sarcina* represented. A fairly large group of gram positive, non-spore forming rods was present. These cultures were not identified since they were very heterogeneous with respect to physiological characteristics and several species were probably represented. Finally, a few cultures of aerobic spore formers were found.

One-hundred and nineteen cultures of bacteria were isolated during the 1956 maple season. Generic classification of these cultures showed 101 (85%) of them to be species of *Pseudomonas;* 5 (4.2%) were *Chromobacterium;* 3 (2.5%) were *Flavobacterium;* 1 (0.8%) *Bacillus;* and 9 cultures (7.6%) were unclassified. The only major difference from the 1955 flora was the absence of *Micrococcus.*

Yeasts from maple tapholes: Nine species representing 5 genera of yeasts were identified among 412 cultures of yeasts isolated during the 1955 season (Table 3). All cultures were found to be non-fermentative, anascosporogenous types. The most fre-

TABLE 1

Origin of cultures isolated from maple tapholes (1955 season)

Tapping date	Trees tapped	Tapholes sampled	Samples taken during season	Number of cultures [1]
January 10	10	20	200	320
January 25	10	20	200	311
February 10	10	20	180	317
February 25	10	20	140	226
March 10	10	20	100	99
Totals:	50	100	820	1273

[1] Isolations were made from all samples in which microorganisms were found.

All plates were incubated at room temperature for 3 to 5 days. Representative colonies from platings of the highest dilutions were transferred to culture tubes containing appropriate media to preserve the cultures for identification studies. Isolations were made from platings of all samples in which microorganisms were found to be present during the 1955 season.

All cultures isolated were divided into 3 major groups (bacteria, yeasts, and molds) on the basis of colonial appearance and microscopic morphology. The bacteria were examined according to procedures found in *Manual of Methods for Pure Culture Study of Bacteria (4),* and identified with reference to *Bergey's Manual (3).* Yeast isolates were further examined and identified according to the system of Lodder and Kreger-Van Rij *(10).* Methods used were as outlined by these authors except that sporulation tests were run with a vegetable juice (V-8) medium *(9),* fermentation and assimilation in peptone-yeast extract broth *(9),* and hyphae production in yeast-morphology agar (Difco). Mold cultures were examined only for morphological characteristics and were identified to genus using Barnett *(1)* and Beneke *(2)* as references.

RESULTS

Population studies of the sap from tapholes demonstrated that both bacteria and yeasts grew in the tapholes. The increase in averages of logarithms of counts per ml. of sap from 30 regular tappings during 1955 is shown in Figure 1. Although the populations of yeasts and molds were not considered separately, it should be noted that molds were rarely found after the first 2 weeks of the season. Therefore, these data did not indicate appreciable mold development in tapholes. However, other data, to be discussed elsewhere, indicate that molds may grow within the tappings and not appear to any extent in the sap.

Bacteria from maple tapholes. The 469 cultures of bacteria isolated during the 1955 season were distributed among 4 major groups (Table 2). As might be expected,

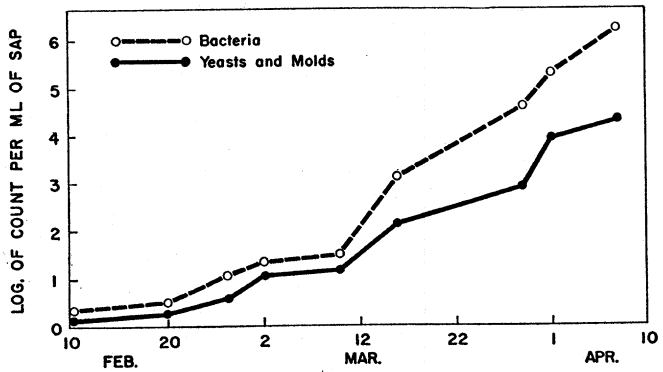

Figure 1. Development of microorganisms in maple tree tapholes as indicated by populations occurring in the sap.

TABLE 3

Yeasts from maple tapholes (1955 season)

Species	Number of cultures	Percent of total
Trichosporon pullulans[1]	84	20.4
Rhodotorula glutinis	75	18.2
Candida sp.	48	11.6
Torulopsis aeria	42	10.2
Cryptococcus laurentii	36	8.7
Cryptococcus diffluens	32	7.8
Cryptococcus albidus	32	7.8
Candida curvata	23	5.6
Torulopsis candida	8	1.9
Unclassified	32	7.8
Totals:	412	100.0

[1] Tentative classification.

quently isolated yeast was one which was tentatively identified as *Trichosporon pullulans*[*]. *Rhodotorula glutinis* was a close second in frequency of isolation. An unidentified species of *Candida* and *Torulopsis aeria* were next in order, followed by 3 species of *Cryptococcus* and *Torulopsis candida*. A fairly large non-uniform group of yeasts remained unclassified.

Fifty-four yeast cultures were isolated during the 1956 studies and were found to be of the same general types. However, the greatest proportion of this collection belonged to the genus *Candida*.

Molds from maple tapholes. The number of molds (370) isolated in 1955 was much out of proportion to their relative numbers in the sap. Practically all of these cultures were obtained early in the season when the total populations of organisms were quite low. The 9 genera found to be represented among these cultures are given in Table 4. *Penicillium* and *Phoma* were the most frequently isolated genera of those identified. However, the majority of the cultures, comprising about 30% of the total, were not identified because of their failure to produce typical fruiting structures on laboratory media. Many of these are probably members of the class *Basidiomycetes*.

There were 22 additional cultures isolated in 1955 which were classed as streptomycetes.

[*] These cultures were identical with the description of this species presented by Lodder and Kreger-Van Rij *(10)* except for the development of a dark (almost black) appearance in old (2-3 weeks) cultures grown on vegetable juice sporulation medium *(9)* or on yeast morphology agar (Difco).

TABLE 4

Molds from maple tapholes (1955 season)

Genus	Number of cultures	Percent of total
Penicillium	83	22.4
Phoma	60	16.2
Hormodendrum	36	9.7
Fusarium	26	7.0
Alternaria	16	4.4
Cephalosporium	13	3.5
Rhizoctonia	11	3.0
Aspergillus	10	2.7
Coniothyrium	6	1.6
Unidentified	109	29.5
Totals:	370	100.0

DISCUSSION

The fact that bacterial populations in sap from maple tapholes are usually greater than the yeasts and molds does not necessarily mean that the former are more important in the stoppage of sap flow. Other experiments in this project have demonstrated that representatives of all 3 groups may result in sap flow stoppage *(12)*. Most yeasts do not develop the high population levels that occur with bacteria. Also, the yeasts may not be as easily washed out of the taphole as the bacteria. It has been demonstrated that molds will grow quite well in the tapholes and result in stoppage of sap flow without any appreciable number appearing in the sap *(12)*. Therefore, it is necessary to consider all organisms capable of growing under these conditions as possible causes of a loss in sap yield.

The microorganisms found in the sap as it comes from the spile are of the same general types known to be responsible for spoilage of sap *(7)*. The *Pseudomonas, Achromobacter,* and *Flavobacterium* groups are common psychrophilic types which are found in water and soil; and, thus, would be expected to develop in conditions such as those encountered in maple tappings. The micrococci and aerobic spore formers might also be anticipated in such an environment.

Trichosporon pullulans has been found in the exudates of birch and chestnut trees, and *Rhodotorula glutinis* has been isolated from air, wood pulp and soil *(10)*. The presence of *Torulopsis aeria, Cryptococcus laurentii, Cryptococcus albidus* and species of *Candida* would not be considered unusual in this environment. Although the presence of *Cryptococcus diffluens* and of *Candida curvata* was somewhat of a surprise to us, these species have been reported by di Menna *(5, 6)* to be inhabitants of soils in New Zealand. In fact, all of the species identified in the present study except *Torulopsis candida* were found in these soils. It is of particular interest that not a single culture of a fermentative or an ascosporogenous yeast was isolated. Wickerham and Burton *(13)* found a number of fermentative species of 3 related ascosporogenous genera associated with trees, and with insects dependent on both the trees and the yeasts. However, some of these were related only to conifers and slime fluxes of deciduous trees.

SUMMARY

Both bacteria and yeast populations were shown to develop to fairly high levels in maple tree tapholes during the sap season. This was demonstrated by their presence in the sap as it flowed from the tree. Conversely, low mold counts were observed in the sap early in the season, but did not develop to high numbers. This does not prove, however, that the molds did not grow in the tapholes.

Over two-thirds of 588 bacterial cultures isolated were gram negative rods of the genera *Pseudomonas, Achromobacter,* and *Flavobacterium. Pseudomonas geniculata* was the most frequently encountered species. Also present were representatives of the genera *Micrococcus, Bacillus, Sarcina,* and *Chromobacterium,* and a heterogeneous group of non-spore forming, gram positive, rod-shaped bacteria.

Four-hundred and forty-six yeast cultures were isolated during the two

seasons; and, without exception, were all anascosporogenous, non-fermentative types. Of 412 cultures studied in detail, *Trichosporon pullulans* (tentative classification) and *Rhodotorula glutinis* were the most frequently isolated. The remaining cultures were about equally distributed among two species of *Candida,* three of *Cryptococcus,* two of *Torulopsis,* and a miscellaneous group of unidentified yeasts.

A group of 370 mold cultures were studied and 9 different genera recognized among them. However, the largest group of mold cultures (approximately 30 percent) were not identified due to their failure to produce typical fruiting structures on laboratory media.

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IDENTIFICATION OF MICROORGANISMS FROM MAPLE TREE TAPHOLES [a, b]

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Recent studies *(11, 12)* have established that the growth of microorganisms in maple tree tapholes results in low sap yields. Naghski and Willits *(11)* indicated that bacteria were the most frequent cause of premature stoppage of sap flow, but that yeasts and molds were involved in some instances. Beyond this, there is no information available on the identity of the microflora in the taphole.

Edson *et al. (7)* made an extensive study of the microorganisms in spoiled sap and demonstrated the presence of various types of bacteria, yeasts, and molds. Bacteria of the genera *Bacillus, Micrococcus* and *Pseudomonas* were found in these saps. One species of the bacilli described by these authors, *Bacillus aceris,* was later reclassified as *Achromobacter aceris (3).* The yeasts from the spoiled saps were described only as true yeasts and yeastlike organisms which formed red or grey colonies. Molds of the genera *Penicillium* and *Aspergillus* were identified. Fabian and Buskirk *(8)* reported *Aerobacter aerogenes* as a causative agent of maple sap spoilage, which resulted in ropy sirup.

This work was conducted as a part of an overall program to determine the influence of microorganisms in maple tree tapholes on sap yields. It was necessary to determine which microorganisms were present before their relative importance could be ascertained.

EXPERIMENTAL

All sap samples collected for this investigation were obtained during the 1955 and 1956 seasons of sap production. Maple trees that had never been tapped were selected for the tapping and sampling studies [d]. These were part of a sugar bush on the campus at East Lansing. All samples were collected from the running tapholes or from the collection containers at intervals throughout the 2 seasons, usually during the major sap runs. Samples of sap were taken from normal commercial type tappings and from tappings which were maintained in a closed system *(12)* in an attempt to prevent microbial contamination. Table 1 indicates the number of trees tapped during the 1955 season and the number of samples taken and cultures isolated according to the date of tapping. A total of 1273 cultures was isolated during the 1955 season and an additional 173 were obtained during the 1956 season.

Populations of bacteria and of yeasts and molds were determined in all samples using standard plating and counting techniques. Nutrient agar was used for the enumeration of the bacteria and dextrose agar containing 0.1% yeast extract, acidified with 2 ml. of 5% tartaric acid per 100 ml. of medium, was used for yeast and mold counts.

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[a] Journal Article No. *2254,* Michigan Agricultural Experiment Station.

[b] This work was conducted under a contract with the Eastern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

[c] Present address: Wisconsin Malting Co., Manitowoc, Wisconsin.

[d] A more detailed experimental outline of the tapping and sampling methods is presented by Sheneman *et al. (12).*

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